The present specification has been objected to for allegedly failing to satisfy the "enablement" requirement of 35 U.S.C. §112, first paragraph. The Examiner appears to be questioning how those skilled in the art would determine from the present specification whether or not the amount of ligand bound to binding agent is an insignificant portion of any analyte present in a sample. The Examiner further characterizes as "misleading" the description of prior art provided in the present application.

Claims 1-9 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. The Examiner's comments with regard to this ground of rejection imply that the Examiner considers that clarification is needed regarding the expressions "loading", "same operation" and "insignificant proportion" as used in claim 1. The Examiner also observes in this regard that in claim 5 the specified range of the liquid sample volume includes zero, which clearly would not work.

Claims 1-11 have been rejected under 35 U.S.C. §103 as allegedly unpatentable over the combined disclosures of applicant's own published international Patent Application No. WO 84/01031 and published UK Patent Application No. 2,099,578A. The Examiner also cites Chang, U.S. Patent No. 4,591,570 as evidence of obviousness. According to the Examiner, it would have been obvious to a person of ordinary skill in the art at the time the present invention was made to simultaneously quantify several analytes using trace levels of antibody in a device such as that described in the Chang patent because it would be expected to work.

Each and every objection and rejection set forth in the May 31, 1991 Official Action is respectfully traversed.

1. No Attempt Has Been Made To Incorporate Essential Subject Matter By Reference To WO 88/01058

The Examiner's position in this regard is based on the erroneous premise that the subject matter of WO 88/01058,

to which reference is made at page 16, lines 17-19 of the present specification, is essential to the practice of the present invention. The present specification provides all of the particulars required for one skilled in the art to practice the claimed invention. A description of the invention of essentially the same scope as claim 1 is found in the paragraph bridging pages 7 and 8 of the specification. Moreover, the detailed description of the invention, together with the working examples, set forth at pages 8-22 of the present specification, provide specific direction concerning the following: (i) choice of a solid support; (ii) choice of a suitable binding agent; (iii) loading of binding agents to the support at plural locations; (iv) contacting the loaded support with test sample of the prescribed volume; and (v) selection and use of site recognition agents for determining fractional occupancy of the binding agents by the analytes. In addition, specific determinations of several representative analytes, namely, TNF and HGC; T_4, T_3 and TSH; and HCG and FSH are detailed in Examples 1-4. Clearly, the disclosure of the invention provided in the present specification is more than sufficient to support the claims and to satisfy the disclosure requirements of §112. Any further details contained in WO 88/01058 may be considered cumulative to the present specification or non-essential. As such, incorporation by reference to WO 88/01058 cannot be considered objectionable.

2. The Present Specification Fully Complies With The Enablement Requirement Of 35 U.S.C. §112

As stated at pages 1 and 2 of the present specification, the fractional occupancy of binding sites on the binding agent by an analyte in the fluid sample being measured is related to the initial concentration of the analyte in that sample by the equation at the top of page 2 when, but only when, the amount of binding agent present is so low that its introduction into the fluid sample causes no significant diminution of the concentration of ambient

analyte. As disclosed at page 1, lines 22-27, the fractional occupancy of binding sites under these conditions is effectively independent of the absolute volume of the fluid and of the absolute amount of binding agent, i.e., independent within the limits of error usually associated with the measurement of fractional occupancy. An analogy may be drawn to the measurement of temperature by means of a thermometer or other temperature-measuring device. The introduction of a temperature-measuring device into a system whose temperature is to be measured must inevitably have an influence on the overall temperature of that system and one must necessarily design the device in relation to the system so that the disturbing influence of the introduction of the device makes no significant difference to the temperature measured. What is "significant" in any particular instance depends on the accuracy with which measurements are able to be made and the error level which is acceptable. In the present instance, it is made clear at page 4, lines 30-31 and page 6, line 34, as well as page 13, lines 1-5, that the insignificant proportion of the analyte is generally less than 10%, usually less than 5% and for optimum results 1-2% or less. These figures give sufficient indication of what is intended.

The Examiner's comments at page 3, lines 2-13 of the May 31, 1991 Official Action are vigorously disputed. The generally recommended practice in the field of immunoassay and immunometric techniques has been to bind substantial amounts of the analyte, optimally about 50% or considerably more depending on the nature of the assay. When such high proportions of analyte are bound, the fractional occupancy of the binding sites on the binding agent by the analyte is dependent not only on the concentration of the analyte but also on the absolute volume of the fluid sample and on the absolute amount of binding agent. In carrying out assays under such circumstances, it is, therefore, necessary to use standard volumes of sample and standard amounts of binding agent so that the figure obtained for fractional occupancy of

the binding sites with a fluid sample of unknown concentration can be compared with figures obtained for fractional occupancy under the same conditions using samples of known concentration. By operating in accordance with the present invention, it is no longer necessary either to ensure that standard volumes of sample are used or to ensure that standard amounts of binding agent are used, as between the unknown sample on the one hand and the samples of known concentration on the other hand. Thus, the user is free to employ a multiple assay system in a manner which could not be done under the older procedure.

In the "Background" of the invention, applicant accurately describes the generally recommended practice over which the present invention provides a distinct operational advantage, as noted above. Applicant's description of the generally recommended practice is not intended to, and indeed does not imply that other methods will not work; it is factually accurate and is in no sense misleading.

3. Claims 1-9 Satisfy The Definiteness Requirement Of 35 U.S.C. §112

The relevant inquiry in determining whether a given claim satisfies the requirements of 35 U.S.C. §112, second paragraph, is whether the claim sets out and circumscribes a particular area with a reasonable degree of precision and particularity. In re Moore, 169 U.S.P.Q. 326 (CCPA 1971). Applicant respectfully submits that with respect to the claims now pending in this application, such inquiry must be answered in the affirmative.

Claim 1 calls for "loading a plurality of different binding agents . . . onto a support means at a plurality of spaced apart locations such that each location has not more than 0.1 V/K moles of a single binding agent . . .." Thus, the meaning of "loading" is perfectly clear from claim 1 itself, without resort to the specification. As plainly required in claim 1, binding agents are loaded onto a support

means at a plurality of spaced apart locations, with each location having a single binding agent. The term "loading", as used in claim 1, cannot be reasonably be read to cover a single location having more than one binding agent thereon.

The Examiner's statement that the "second step of the method reads on samples in individual wells as well as many samples in one well" is not understood. The second step of claim 1 requires that the loaded support means is contacted with the liquid sample to be analyzed such that each of the spaced apart locations bearing the different binding agents is contacted in the same operation with the liquid sample. As can be seen in Example 1, a test device for use in practicing the method of claim 1 can be prepared having distinct binding agents applied to separate spots or locations in the well of a microtiter plate. In carrying out the method of the invention, as described in Example 2, the liquid test sample containing a plurality of analytes of interest is added to the well containing the plurality of binding agents at spaced apart locations. It is certainly within the scope of this invention to provide a plurality of other different binding agents in other wells of the microtiter plate for testing the same or other liquid samples. In no case would different samples be mixed as applied to the spaced apart locations bearing the different binding agents, as mixing of different samples, e.g., body fluids from two patients, would defeat the assay.

In the event the Examiner repeats this ground of rejection, it is respectfully requested that further explanation be provided regarding the basis for the statement that the second step of claim 1 reads on samples in individual wells as well as many samples in one well.

The expression "in the same operation" is believed to be clear on its face. In the step of contacting the loaded support means with the liquid sample to be analyzed, the liquid sample is applied to the support means in such a way that each of the spaced apart locations is effectively

contacted simultaneously with the liquid sample. As is readily apparent from reading examples 2, 3 and 4 of the present application, the spaced apart locations of binding agent on the support means are contacted with the liquid sample to be analyzed in the same operation by applying to the support means drops of the test sample which flow over the spaced apart locations of the binding agents at substantially the same time. Alternatively, the support means could be dipped into the test sample to effect contact between the spaced apart locations of binding agent on the support means and the liquid sample in the same operation. In any case, when claim 1 is read in light of applicant's specification, it is quite apparent what is meant by "the same operation".

The cases are legion in which expressions of degree such as "insignificant proportion" have been found to be in compliance with the definiteness requirement of 35 U.S.C. §112. See, for example, Ex parte Martinek, 159 U.S.P.Q. 696 (Bd. Apps. 1967) ("small increments"); and Ex parte Freeman, 100 U.S.P.Q. 15 (Bd. Apps. 1953) ("minor amount"). The rationale which warranted the use of such expressions of degree in Martinek and Freeman clearly support the use of the expression "insignificant proportion" in applicant's claim 1.

In reversing an indefiniteness rejection of claims directed to a method of preparing a fluid dispersion in Martinek, the Board stated, at 697:

> The rejection of claims 12-18 as vague and indefinite in "small increments" and "increments" and "an abrupt decrease in the consistency" will not be sustained. These expressions are sufficiently descriptive of the manipulation involved and it is not feasible to put quantitative limits thereon because these would vary with the particular materials employed.

Likewise, in Freeman, where the applicant claimed a method of hardening steel, the Board stated at 316:

> We are unable agree with the Examiner that the expression "minor amounts" and "major amounts" render the claims indefinite, because the meaning of these expressions is quite clear when they are read in the light of the specification. While these expressions are broad, they do not render the claims subject to a rejection on the ground that they fail to define the invention with the particularity required by Sec. 4888 R.S. [the forerunner of 35 U.S.C. §112] because it is quite clear from the disclosure in the present case that specific proportions of the oil and the sulfonate are not critical and that the invention does not reside in these proportions [Bracketed comment added].

Similar reasoning compels the conclusion that the expression "insignificant proportion", as used in claim 1 herein, is sufficiently definite to satisfy the requirement of §112, second paragraph.

Turning to claim 5, it should be noted that claim 5 is dependent from claim 1. As such, claim 5 must be construed to incorporate by reference all the limitations of claim 1. 35 U.S.C. §112, fourth paragraph. Claim 1 specifically calls for contacting the loaded support means with the liquid sample to be analyzed. Thus, claim 5, when properly interpreted to include all of the limitations of claim 1, cannot reasonably be read as including zero volume, since to do so would effectively read the contacting step out of claim 1. Cf. In re Kirsch, 182 U.S.P.Q. 286 (CCPA 1974).

In summary, applicant's position with respect to the rejection of claims 1-9 based on 35 U.S.C. §112, second paragraph, is that any person skilled in the art having applicant's disclosure and claims before him or her, would be

possessed of a reasonable degree of certainty as to the exact subject matter encompassed within the claims. Nothing more is required under §112, second paragraph.

4. The Disclosures Of The Cited Prior Art References Do Not Render The Claimed Subject Matter Obvious

Before addressing the obviousness argument advanced by the Examiner in the May 31, 1991 Official Action, a brief discussion of the present invention may be helpful for the purpose of pointing out those aspects of the invention which applicant deems to constitute patentable distinctions over the prior art.

The Examiner's position regarding the alleged obviousness of the present invention reflects a fundamental misapprehension of the extent to which the methodology embodied in the present invention contravenes currently accepted views of immunoassay design. Indeed, many experienced immunologists, when first introduced to the methodology of the present invention cannot immediately understand why it is that amounts of analyte vastly in excess of the amount of antibody in the system do not result in total occupancy of all antibody binding sites and that the fractional occupancy of these sites serves as a measure of analyte concentration.

It is generally understood that the so-called competitive assays rely on competition between labelled and unlabelled analyte molecules for a limited number of binding sites, which are present in a concentration approximating 1/K (K being the equilibrium constant of the binding agent for the analyte) to yield maximal sensitivity, as recommended by Berson and Yallow ("Methods and Investigative and Diagnostic Endocrinology (1973)), thus implying that 50% of the labelled analyte is bound to antibody at zero unlabelled analyte concentration. Accordingly, analyte concentration is determined on the basis of distribution of labelled analyte

between antibody bound and free fractions. This common perception of the way in which competitive assays work generally assumes that virtually all antibody binding sites are occupied, even when no unlabelled analyte molecules are present in the system, and only labelled analyte molecules are present.

In the case of so-called non-competitive assays relying on the use of labelled antibodies, the amounts of labelled antibody used in previous techniques are much higher than those used in competitive assays, being in substantial excess in comparison with the concentration of analyte present. This is generally considered to be the reason for the higher sensitivity of non-competitive compared with competitive methods, as well as for the much shorter incubation times that non-competitive assays require.

The primary advantages of the assay method of the present invention are that: (a) it uses far less antibody than is conventional (particularly in non-competitive assays, which is of considerable advantage to a manufacturer), (b) its sensitivity is at least equal to, and in principle greater than, that obtainable using conventional assays, and (c) that this high sensitivity can be achieved using incubation times that are as short, and, in principle, shorter than other conventional approaches. The latter advantages are unexpected and have never previously been recognized. Indeed, a common criticism of the method of the invention is that because the amount of antibody is so small, incubation times must necessarily be greatly prolonged in order to reach thermodynamic equilibrium.

Experimental studies (backed by computer analysis) have demonstrated that this common belief is unsound. Operating under conditions disclosed in the present specification, fractional occupancy of the antibody is a measure of the signal/noise ratio since the "signal" is emitted by "developing" antibody attached to these sites, while the noise is proportional to the total number of sensor

antibody sites attached to the solid support. The higher the signal noise ratio, the higher the sensitivity of the system. The two graphs of Exhibit I, which is submitted herewith, show computer plots of the fractional occupancy of the sensor antibody as a function of incubation time (represented as $1/k_d$, where $k_d=0.693/T_{1/2}$, i.e., the sensor antibody/analyte dissociation rate constant, in which $T_{1/2}$ is the half dissociation time) for two different sensor antibody concentrations (i.e., 10/K, lower graph, and 0.001/K, upper graph). Thus, 10,000 fold more antibody is used in the lower assay system as compared with the upper. But at all incubation times, the fractional occupancy of the lower amount of antibody (i.e., the signal/noise ratio) is greater than that of the larger. In other words, contrary to common beliefs, the assay method of the present invention is capable of being performed faster and with greater sensitivity as compared with prior art assay techniques.

From the foregoing, it should be appreciated that the assay of the present invention provides previously unrecognized advantages in regard to sensitivity and speed, coupled with the ability to measure accurately many different analytes simultaneously in the same sample.

Because the prior art of record fails to teach or suggest the essential aspects of applicant's invention and its attendant advantages, as briefly outlined above, the cited prior art does not provide a proper basis for rejecting applicant's claims, as the following discussion will clearly demonstrate.

Turning to the Examiner's allegation of obviousness based on the disclosures of WO 84/01031, UK 2,099,578A and Chang, it is settled law that the burden of establishing a *prima facie* case of obviousness falls upon the Examiner. Ex parte Wolters, 214 U.S.P.Q. 735 (Bd. Apps. 1979). In determining whether a case of *prima facie* obviousness exists, it is necessary to ascertain whether or not the disclosures of the cited prior art would appear to be sufficient to one of

ordinary skill in the art to make the claimed substitution, combination or other modification. In re Lalu, 223 U.S.P.Q. 1257 (Fed. Cir. 1984). Merely because it is possible to find several prior art disclosures which might be combined in such a way as to arrive at the claimed subject matter, it does not make the combination of disclosures obvious unless the art also contains something to suggest the desirability of the proposed combination. In re Imperato, 179 U.S.P.Q. 730 (CCPA 1973). In the present case, there is nothing to suggest the desirability of combining the disclosures of WO 84/01031, UK 2,009,578A and Chang in the manner proposed by the Examiner.

As compared with the assay disclosed in applicant's WO 84/01031, claim 1 of the present application requires, as an essential aspect, that the number of moles (N) of binding agent present at each spaced apart location on the support means, the volume (V) of the sample, and the equilibrium constant (K) of the binding agent for the analyte are related as set out in claim 1, namely, $N<0.1\ V/K$. This relationship is nowhere to be found in the prior art of record and constitutes an essential inventive aspect of applicant's assay method. It has long been held that all claim recitations must be considered in determining patentability under 35 U.S.C. §103. In re Saether, 181 U.S.P.Q. 36 (CCPA 1974). Indeed, it is error to ignore specific recitations distinguishing over the prior art. In re Glass, 176 U.S.P.Q. 489 (CCPA 1973).

UK 2,099,578A and Chang, which are cited in combination with WO 84/01031 in rejecting claims 1-11 clearly fail to make up for the fundamental deficiency noted above in the disclosure of WO 84/01031. Rather than provide the critical insight regarding the relationship $N<0.1\ V/K$, which is required for those skilled in the art to bridge the unmistakable gap between the disclosure of WO 84/01031 and the present invention, the disclosures of UK 2,099,578A and Chang actually teach away from the present invention.

As acknowledged at pages 3 and 4 of the present specification, UK 2,099,578A allows a plurality of

quantitative immunoassays to be performed on the same support. However, the number of moles of antigen present at each location on the support, according to the disclosure of UK 2,099,578A, is such as to bind essentially all of the analyte present in the liquid sample under test. This is evident from the fact that the quantitative method there described involves calibration with known amounts of immunoglobulin being applied to the support. The disclosure of UK 2,099,578A thus points away from any combination with applicant's WO 84/01031 that would lead to the present invention.

Chang adds little if anything of relevance which would tend to lead those skilled in the art to the assay method of the present invention. Chang discloses an immunoassay device in which an array of antibody coating spots is used for the qualitative determination of whether certain antigens are present in a particular sample. Different antibodies are attached to each of the spots and the user then simply notes which of the antibody spots bind antigen in the samples under test. There is no quantitative determination of analyte concentration such as is achievable by the present invention.

Although the foregoing discussion has been directed toward applicant's assay method, as defined in claim 1, claims 2-9 which depend directly or indirectly from claim 1 are likewise patentable for the same reasons advanced with respect to claim 1. In re Fine, 5 U.S.P.Q. 2d 1596 (Fed. Cir. 1988). Claims 10 and 11 drawn, respectively, to a device and kit for use in practicing the assay method of claim 1 are also distinguishable from the prior art of record in that both of claims 10 and 11 call for a solid support means having located thereon a plurality of different binding agents at a plurality of spaced apart locations with each location having not more than 0.1 V/K moles of a single binding agent. In this respect, the device of claim 10 and the kit of claim 11 are clearly patentably distinguishable over the prior art of record.

Inasmuch as the prior art references cited in support of the §103 rejection of claims 1-11 fail to teach or suggest the claimed matter as a whole, it necessarily follows that the prior art does not render applicant's invention *prima facie* obvious. Accordingly, the rejection of claims 1-11 under 35 U.S.C. §103 based on the combined disclosures of WO 84/01031, UK 2,099,578A and Chang is improper and should be withdrawn.

As further evidence of the patentability of the present invention, the Examiner's attention is respectfully directed to the International Preliminary Examination Report (copy attached) issued in the underlying PCT application. In the International Preliminary Examination Report the original claims were determined to satisfy all of the patentability criteria and, thus, deemed to be patentably distinguishable over WO 84/01031 and UK 2,099,578A, which were among other prior art citations included in the PCT International Search Report.

In view of the foregoing remarks, it is respectfully urged that the objection and rejections set forth in the May 31, 1991 Official Action be withdrawn and that this application be passed to issue, and such action is earnestly solicited.

Respectfully submitted,

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Enclosure: - Exhibit I
- PCT/GB88/00649 International Preliminary Examination Report